Department of Cellular Pathology

Timothy J. O'Leary, M.D., Ph.D.
Chairperson
Date of Appointment - 21 January 1987

STAFF

Danny Urquhart, Research Administrator
Anna Thomas, Secretary

In addition to his usual administrative responsibilities, Dr. O'Leary serves on the editorial board of two journals, serves as the chairholder of the National Committee on Clinical Laboratory Standards subcommittees on molecular hematology and immunocytochemistry, as vice-chairholder of the NCCLS Molecular Methods Area Committee, as consultant to the Food and Drug Administration, as attending pathologist at the National Institutes of Health, and as director for the USCAP course "Tumor Prognosis." He lectures frequently at national and international courses, conferences, and symposia.

One major administrative/scientific accomplishment has been in the area of funding. Departmental funding has increased from zero in 1987, to almost $1 million this year. Research and educational activities include the use of classical, quantitative, and molecular methods to predict tumor prognosis and research on the structure of biological membranes. Dr. O'Leary participates in the diagnostic activities of the Cytopathology and Molecular Pathology Divisions.

QUANTITATIVE PATHOLOGY

Robert L. Becker, Col, USAF, MC, Associate Chairperson and Chief, Quantitative Pathology
William Oliver, LTC, MC, USA, Forensic Pathlogist
Ulrika Mikel, M.G.A., Cytologist
Nina Sweeney, M.S.E.E., Computer Scientist
Lena Hohmann, M. Pharm, Biologist
Joseph Griffin, Ph.D., Biologist
Annette Geissel, M.S., Biologist
Elzbieta Kaczmarek, Ph.D., Callender-Binford Fellow
Ruixia Zhao, Computer Scientist

The division's responsibilities include flow cytometry (FCM) and image analysis. FCM is staffed by Dr. Griffin with help from Ms. Geissel and Ms. Hohmann and primarily involves ploidy and S-phase analysis of paraffin-embedded tissue for consultation and research. Other staff have a broad mission to develop and apply computer-assisted techniques extracting information from pathology images. Activities include quantitative microscopy, forensic image analysis, artificial intelligence, and scientific computing. Applications in practice and under development include image analysis of cell ploidy and growth, patterned injury analysis, and three-dimensional visualization/analysis of cells, tissues, and forensic images. The division has performed seminal work in three-dimensional visualization of thick tissue sections and in forensic image analysis, resulting in formal commendation by senior military commanders and by the Attorney General of the United States. Division staff are extensively tasked to assist development of computer-oriented imaging applications throughout the Institute,

particularly those involving wide-area networking.

CYTOPATHOLOGY

M. Tellado, LtCol, USAF, MC, Cytopathologist and Acting Chief, Cytopathology
S. Buckner, M.S., Senior Cytotechnologist, Consultation Service
I. Ali, M.S., Chief, Cytotechnologist, Screening Laboratory
E. Delgado, M.S., Senior Cytotechnologist, Screening Laboratory
R. McNeill, M.S., Senior Cytotechnologist, Screening Laboratory
A. Stevens, M.S., Junior Cytotechnologist, Screening Laboratory
C. Brooker, Msgt, USAF, Superintendent, Accessioning and Quality Assurance
P. Edgar, Secretary
A. Reeder, Accessioning technician
R. Matthres, Accessioning technician

Work includes consultation on difficult cases, primary screening of approximately 40,000 cases per year (primarily Air Force, but also Navy and PHS), research on automated rescreening and telecytology, training for military and PHS residents, an annual course, and a joint cytopathology fellowship program with the Johns Hopkins University.

Cytologic examination is increasingly important as a definitive diagnosis technique; development of a strong, nationally recognized presence in cytopathology is the focus of the department's most intensive developmental activity.

BIOPHYSICS

J. Mason, Ph.D., Chief, Biophysics
M. Batenjany, Ph.D., Postdoctoral Fellow
R. Cunningham, M.S., Chief, Flow Cytometry Laboratory

Work includes basic research on membrane structure and the effects of toxins on membranes and membrane proteins, and use of biophysical methods for pathologic diagnosis. Highlights include the development of immunoliposomal PCR and the development of preparation methods for infrared and Raman microspectroscopy of tissue, which have now been adopted worldwide.

MOLECULAR PATHOLOGY

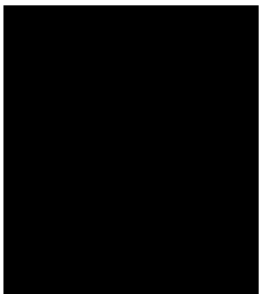
J. Taubenberger, M.D., Ph.D., Chief, Division of Molecular Pathology
J. Lichy, M.D., Ph.D., Director, Molecular Diagnostics Laboratory
A. Krafft, Ph.D., Chief Medical Technologist
B. Duncan, B.S., Medical Technologist
H. Diebert, M.T., Medical Technologist
C. Wright, Ph.D., Senior Investigator
T. Fanning, Ph.D., Senior Investigator
A. Tatro, Ph.D., Postdoctoral Fellow
A. Hubbs, Ph.D., Postdoctoral Fellow
G. Brown-Stephano, Ph.D., Postdoctoral Fellow
A. Reid, M.S., Biologist
M. Tsai, M.S., Biologist
M. Zavar, B.S., Biologist, ARP
M. Majidi, M.S., Biologist, ARP

The major diagnostic activity is operation of the Molecular Diagnostics Laboratory. This laboratory will conduct approximately 6,000 assays on 600 formalin-fixed, paraffin-embedded cases this fiscal

year and is recognized as a national leader in this area. Basic research includes transposable elements in cancer, molecular pathobiology of breast cancer, transcriptional regulation in viruses, tumor suppressor gene function, and development of the hematopoietic system. Applied research ranges from the molecular epidemiology of morbilliviruses in dolphins to the diagnosis of the (2;5) translocation in anaplastic large cell lymphoma. This division accounts for the bulk of the department's grant funding, which comes from such diverse sources as the Army Medical Research and Development Command, the National Institutes of Health, and others. Dr. Taubenberger also serves as an attending pathologist at the National Institutes of Health.

OTHER INTERESTING FACTS

Department staff (average age < 40) has published over 250 refereed papers.



DIVISION OF BIOPHYSICS

Jeffrey T. Mason, Ph.D.
Chief
Date of Appointment - 1 January 1993

MISSION

The mission of the division is to develop new knowledge and techniques in basic and applied molecular biology through the application of biochemical, biophysical, and chemical methods to the study of biological systems. Techniques utilized include flow cytometry, organic synthesis, infrared and Raman spectroscopy, scanning and titration calorimetry, fluorescence spectroscopy, x-ray diffractometry, and atomic force and electron microscopy.

ORGANIZATION

STAFF

Scientific

(A) Michael M. Batenjany, Ph.D.
Robert E. Cunningham, M.S.
Jeffrey T. Mason, Ph.D.
Timothy J. O'Leary, M.D., Ph.D.

RESEARCH

Research activities during the past year include developmental work on immunoliposome-PCR (an ultrasensitive assay system for biological and chemical antigens); continuing studies designed to understand the structure and function of biological membranes and the interaction of these systems with extrinsic molecules, such as alcohols and anesthetics; and the influence of alterations of membrane lipid structure and pressure on the function of integral membrane proteins, such as acetylcho-

line receptor. Other research activities included the coordination of histology and flow cytometry on breast cancer cases in conjunction with the Department of Cellular Pathology breast cancer project.

EDUCATIONAL ACTIVITIES

1. One-day course taught in house on in situ hybridization techniques (R. E. Cunningham).
2. One-day course taught at Catholic University on flow cytometry. (R. E. Cunningham).

CONSULTATION ACTIVITIES

Consultation on four JC virus cases by performing in situ hybridization (R. E. Cunningham).

GRANTS FUNDED

The Role of Platelet-Activating Factor in Modulating Biological Membrane Function.

Principle Investigator: Jeffrey T. Mason, Ph.D.

PUBLICATIONS

1. Mason JT, Cunningham RE, O'Leary TJ. Lamellar-phase polymorphism in interdigitated bilayer assemblies. *Biochim Biophys Acta.* 1995;1236:65-72.
2. Mason JT, Cunningham RE, O'Leary TJ, Batenjany MM. Lamellar phase polymorphism in interdigitated bilayer assemblies. *Biophys J.* 1995;68:212a.
3. Batenjany MM, Parrett C, O'Leary TJ, Mason JT. The effect of ethanol on the bilayer properties of the N-methylated series of 1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine. *Biophys J.* 1995;68:430a.

In addition, three items are in press.

[redacted]

DIVISION OF CYTOPATHOLOGY

Miguel V. Tellado, LtCol, USAF, MC
Chief
Date of Appointment - 1 April 1995

Curtis W. Ollayos, LCDR, MC, USN
Staff Pathologist
Date of Appointment - 1 April 1995

MISSION

To provide consultation, education, and research in diagnostic cytopathology.

STAFF

Medical

Timothy J. O'Leary, M.D., Ph.D.
Miguel V. Tellado, LtCol, USAF, MC

(A) Curtis W. Ollayos, LCDR, MC, USN
Carol Adair, LTC, MC, USA
Lester Thompson, LT, MC, USNR

Medical Support

Sally-Beth Buckner, B.S., SCT(ASCP), IAC
Izzat S. Ali, B.S., CT(ASCP), IAC
Angelica B. Stevens, B.S., M.T., CT(ASCP)
Emma Delgado-Cintron, B.S., CT(ASCP)

Administrative

Anna L. Thomas, Secretary
Patricia Edgar, Secretary

CONSULTATION

Cases

Description	*Received*	*Reported*
Surgical	949	949
Military/Federal	718	718
Intramural	338	338
Civilian	231	231

The majority of specimens were exfoliated nongynecologic material and fine-needle aspirations. Specimens are sought that provide either unstained cytologic material or cell blocks for research investigation.

The Air Force Cytology Laboratory, established in 1992 to handle gynecologic specimens that cannot be evaluated at Air Force cytocenters in a timely manner, is operating smoothly, but the caseload is smaller than expected.

Cases

Description	*Received*	*Reported*
Air Force Pap Smears	35,209	35,209

RESEARCH

Dr. Timothy O'Leary, Dr. Miguel Tellado, Dr. Curtis Ollayos, Sally-Beth Buckner, Izzat Ali, and Angelica Stevens are currently involved in a study of "automated rescreening of Pap smears."

Dr. Curtis Ollayos initiated a project on HPV analysis in papillary squamous cell carcinoma of the cervix.

EDUCATION

The week-long Exfoliative and Fine-Needle Aspiration Cytology Course was held June 5-9, 1995. Fifty registrants received a total of 1,800 person-hours of instruction.

GOALS

Major efforts will be aimed at strengthening the research effort, particularly in molecular biologic approaches to solving cytopathologic problems.

PRESENTATIONS

1. February 1995: One and one-half-hour lecture on atypical squamous cells of undetermined significance and atypical glandular cells of undetermined significance.
2. March 1995: One and one-half-hour lecture on atypical squamous cells of undetermined significance and atypical glandular cells of undetermined significance.
3. April 1995: Two-hour lecture on atypical squamous cells of undetermined significance and atypical glandular cells of undetermined significance and nongynecologic exfoliative cytology.
4. June 1995: One-hour lecture on atypical squamous cells of undeteremined significance and atypical glandular cells of undetermined significance.
5. September 1995: One and one-half-hour lecture on atypical squamous cells of undetermined significance and atypical glandular cells of undetermined significance.
6. A lecture to the AFIP staff entitled "The Cytologic Diagnosis of Proliferative Breast Disease."

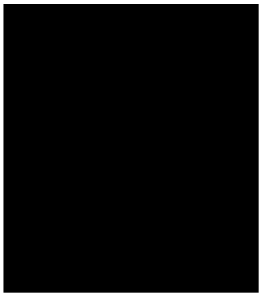
PUBLICATIONS

1. Pap smear abnormalities in the military recruit population. *Mil Med.* November 1995.
2. Marsella RC, Buckner SB, Bratthauer GL, O'Connor DM, Protor J, O'Leary TJ. Identification of genital herpes simplex virus infection by immunoperoxidase staining: comparison with culture results and cytology. *Applied Immunohistochemistry*. 1995;3:184-189.
3. Liaw KL, Hsing AW, Chen CJ, Schiffman MH, Zhang TW, Hsieh CY, Greer CE, You SL, Huang TW, Wu TC, O'Leary TJ, Seidman J, Meinert CL, Manos MM. Human papillomavirus and cervical neoplasia: a case-control study in Taiwan. *Int J Cancer.* 1995;62:565-571.

In addition, one article is in press.

EXTRAMURAL

1. Mrs. Sally-Beth Buckner served as a member of the Cytotechnology Examination Committee of the ASCP Board of Registry.
2. Mrs. Buckner served as a member of the Scientific Program Committee of the American Society of Cytopathology and was director of the panel luncheons for the annual meeting in New York City.
3. Mrs. Buckner reviewed an article for *Laboratory Medicine*.
4. Dr. Curtis Ollayos was appointed assistant clinical professor of pathology, USUHS. He taught multiple sessions to second-year medical students.



DIVISION OF MOLECULAR PATHOLOGY

Jeffery K. Taubenberger, M.D., Ph.D.
Chief
Date of Appointment - 1 January 1994

MISSION

To perform consultation, research, and education in molecular biology and molecular pathology; to develop new techniques for consultative diagnostic molecular pathology and molecular medicine; to explore new areas of molecular biology and determine which may be useful for current or

future development at the Institute; and to collaborate with other CAP departments by performing research using molecular techniques.

STAFF

Principal Investigators

Thomas G. Fanning, Ph.D.
Jack H. Lichy, M.D., Ph.D.
Jeffery K. Taubenberger, M.D., Ph.D.
(D) Cynthia F. Wright, Ph.D.

Scientific Staff

(A) Greg L. Borchert
(A) Beverly Duncan, M.T (ASCP)
Alan E. Hubbs, Ph.D.
Amy E. Krafft, Ph.D., M.T. (ASCP)
Mourad Majidi, M.S.
(A) Wayne T. Matten, Ph.D.
(A) Holly Mentzer-Dibert, M.T. (ASCP)
(A) Corina A. Pambuccian, M.D.
Ann H. Reid, M.A.
(D) Gilda Alves Stephano, Ph.D.
Alicia V. Tatro, Ph.D.
Mark M. Tsai, M.S.
Maryam Zavar, M.S.
(D) Xumei Zhao, M.S.

CONSULTATION

The Molecular Diagnostics Laboratory received 589 cases in consultation in 1995. These cases were received from 15 CAP departments, and on average, three different tests were requested per case. This resulted in 1,401 separate molecular pathology assays completed in 1995.

Dr. Taubenberger and Dr. Lichy participated in the sign out of surgical pathology cases.

RESEARCH

The role of LINE-1 in human cancer is under investigation. The LINE-1 element is a "jumping gene" that causes disease in humans by inserting into important regulatory and structural genes and inactivating them. Our assumption is that LINE-1 elements are not active in normal cells, but are active in malignant cells, and this activation may trigger or enhance the malignant state. Our goals are to: (1) identify and clone LINE-1 elements that are active in breast cancer cells and (2) identify and clone genes that are targets of LINE-1 retrotransposition events.

Studies on the function and regulation of the novel signal transduction proteins encoded by the human ST5 gene have revealed two activities that provide new insights into the function of the gene products. The ST5 protein designated p70 has been shown to have the ability to transform NIH 3T3 cells. The other two ST5 gene products, p82 and p126, lack this function. Current work aims to characterize in more detail the transforming and transactivating activities of the ST5 gene products. Studies of the ST5 enhancer element have been undertaken. Mutational analysis has localized the regulatory elements critical for activity to a 16 bp region within the 330 bp enhancer fragment. Analysis of the specific factors that interact with this region are underway. Antisera have been generated against six segments of the ST5 protein expressed in bacteria.

The novel antigen, LIP-6, and the hematopoietic cells that express it are being characterized in research defining early aspects of lymphocyte and leukocyte differentiation. LIP-6 protein has been immunoprecipitated, and protein sequence information will be used to screen cDNA libraries for the LIP-6 gene. Using LIP-6 in four-color cell sorting, two hematopoietic precursor populations have

been identified, a B cell-committed precursor cell and a multipotential precursor population capable of differentiation into B lymphocytes, T lymphocytes, and cells of the myeloid lineage. Functional and molecular indices of lineage commitment in these cells are being investigated. In other studies, molecular characterization of primitive LIP-6-positive thymocyte cell lines was performed to model the earliest phases of T-cell development. These cell lines, derived from mouse fetal thymus organ culture, are all CD3 negative, CD4 negative, and CD8 negative by surface analysis. However, RT-PCR and T-cell receptor rearrangement assays have been employed to develop a model for the order in which T cell-specific genes are activated in triple negative thymocytes.

Previously, the transcription of vaccinia virus late genes was known to require at least three accessory proteins in addition to a virally encoded RNA polymerase. The genes for those accessory proteins have been mapped to the vaccinia genome, cloned, and expressed in baculovirus. Using the recombinant proteins, we have now determined that two additional factors participate in transcription. The genes for these proteins are currently being sought. In addition, we have tentatively identified one of the recombinant proteins as having interactions with the consensus vaccinia late promoter motif. This interaction is being characterized by DNaseI protection analysis. Other lines of study are directed at finding out which of the accessory proteins interact with the polymerase. Thus far, we have established that the polymerase required for late gene transcription is distinct from that involved in the transcription of early genes.

Loss of heterozygosity (LOH) is being investigated as a marker for genetic changes during breast cancer progression. A study is underway to microdissect intraductal, invasive, metastatic, and recurrent components from approximately 200 ductal carcinomas, sectioned on glass slides. Polymerase chain reaction-based assays are then being performed on DNA isolated from the microdissected cells. This method will allow correlation of genetic changes with histologic stages of progression. Reliable methods for microdissection and DNA analysis from the microdisected tissue have been developed. Five loci at chromosome 11p15 were analyzed for LOH in 50 tumors. LOH was observed in 40% of the cases. When LOH was observed at 11p15, it was usually present in the intraductal as well as the more advanced components of the tumor, consistent with the hypothesis that LOH at this locus occurs during early carcinogenesis.

Bottlenose dolphin specimens from the 1993 Gulf of Mexico and the 1987 Atlantic coast epizootics were analyzed in a collaborative project with the Department of Veterinary Pathology for the presence of morbillivirus. A study in which amplified segments of the morbillivirus genome from these specimens were sequenced was performed. Sequence information was derived from 37 cases, and both cetacean morbilliviruses were identified. The Atlantic coast cases had both the dolphin and porpoise morbilliviruses, while only the porpoise virus was identified in the Gulf of Mexico cases.

Sensitivity and specificity studies were performed on the PCR-based immunoglobulin heavy chain rearrangement assay performed by the Molecular Diagnostics Laboratory on formalin-fixed, paraffin-embedded tissue sections. The overall sensitivity was 68% (a monoclonal rearrangement detected in a B-cell lymphoma), with a specificity of 95%. The lower limit of detection was determined to be the DNA equivalent of 8 to 10 cells. The relative lower limit of detection of a monoclonal band with a polyclonal background was determined to be 20% of the population.

Sensitivity and specificity studies were performed on the PCR-based T-cell receptor gene rearrangement assay performed by the Molecular Diagnostics Laboratory on formalin-fixed, paraffin-embedded tissue sections. The overall sensitivity was 61% (a monoclonal rearrangement detected in a T-cell lymphoma), with a specificity of 95%. The lower limit of detection was determined to be the DNA equivalent of 4 to 8 cells. The relative lower limit of detection of a monoclonal band with a polyclonal background was determined to be 20% of the population.

Sensitivity and specificity studies were performed on the PCR-based t(14;18) translocation assay performed by the Molecular Diagnostics Laboratory on formalin-fixed, paraffin-embedded tissue

sections. The overall sensitivity was 92% (a translocation observed in a follicular lymphoma), with a specificity of at least 94%. The lower limit of detection was a detectable translocation in a dilution between 1:1000 and 1:10,000.

Eighteen cases of follicular lymphoid lesions of the GI tract were analyzed for immunoglobulin heavy chain rearrangement status and for the presence of the t(14;18) translocation in a collaborative study with the Departments of Hematologic and Lymphatic Pathology and Gastrointestinal Pathology. Monoclonal rearrangements were observed in six cases, and t(14;18) translocations were observed in two cases.

Thirty-four cases of Ki-1+ large cell anaplastic lymphoma were analyzed for the t(2;5) translocation by RT-PCR from formalin-fixed, paraffin-embedded material in a collaborative study with the Department of Hematologic and Lymphatic Pathology.

Five cases of Ki-1+ large cell anaplastic lymphoma of the lung were analyzed for the t(2;5) translocation by RT-PCR, for gene rearrangements, and for EBV from formalin-fixed, paraffin-embedded material in a collaborative study with the Department of Pulmonary and Mediastinal Pathology.

Eight cases of lymphoblastic lymphoma were analyzed for immunoglobulin heavy chain and T-cell receptor rearrangements and EBV from formalin-fixed, paraffin-embedded material in a collaborative study with the Department of Hematologic and Lymphatic Pathology.

Twelve corneal transplant biopsy specimens were analyzed for herpes simplex type I in a research study with the Department of Ophthalmic Pathology. Two of the cases were positive.

Thirty-five cases of lymphoid lesions of the orbit were analyzed for immunoglobulin gene rearrangement in conjunction with histologic and immunophenotypic analyses in collaboration with the Department of Ophthalmic Pathology. Monoclonal rearrangements were detected in six cases. The use of this assay in conjunction with histologic and immunophenotypic data is important in the diagnosis of malignant lymphoma of the orbit.

EDUCATION

Dr. Lichy initiated a rotation in molecular pathology for WRAMC pathology residents. In 1995, four residents were trained.

A lecture on molecular pathology techniques was presented by Dr. Fanning at the 5th Annual AFIP Anatomic Pathology Review and Update Course.

Two lectures on the molecular pathology of neoplasia and infectious diseases were presented by Dr. Taubenberger at the 5th Annual AFIP Anatomic Pathology Review and Update Course.

Dr. Lichy presented a clinical staff conference in November 1995 on signal transduction in neoplasia.

Dr. Fanning is currently training Drs. Alicia V. Tatro and Wayne T. Matten, both postdoctoral fellows.

Dr. Taubenberger is currently training Dr. Corina Pambuccian, a postdoctoral fellow.

Dr. Wright is currently training Dr. Alan Hubbs, a postdoctoral fellow.

OTHER ACTIVITIES

Dr. Krafft, Ms. Duncan, and Ms. Mentzer-Dibert attended the 3rd Annual Molecular Diagnostics in Pathology Course offered by the Universities Associated for Research and Education in Pathology, November 1995, in St. Paul, Minnesota.

Dr. Fanning and Dr. Tatro attended the mid-Atlantic transposable element meeting in Washington, D.C.

Dr. Taubenberger served as chairman of the CAP Molecular Pathology Committee. Dr. Lichy served on the Molecular Pathology Committee of the AFIP. Dr. Taubenberger served on the Quality Assurance Committee of the AFIP. Dr. Wright served on the Research Committee of the AFIP.

The division participated in four CAP surveys in 1995. Two surveys involved the analysis of test specimens by molecular methods for clonal expansions of T Cells and B Cells, as well as for chromosomal rearrangements characteristic of specific subtypes of leukemias and lymphomas. Two surveys involved the analysis of test specimens for the presence of viruses. These specimens were tested for the presence of HPV, Epstein-Barr virus, CMV, and varicella zoster virus.

GRANT SUPPORT

"Role of LINE-1 Retrotransposons in Human Breast Cancer," funded by the U.S. Army R&D Command ($69,400 for FY95).

"Function of LINE-1 Retrotransposons in Human Breast Cancer," funded by the Council for Tobacco Research ($45,000 for FY95).

"LINE-1 Retrotransposons as Mutagens in Human Breast Cancer," funded by the U.S. Army R&D Command ($74,564 for FY95).

"The Human ST5 Gene in Signal Transduction and Cancer," funded by the NIH ($108,878 for FY95).

"Characterization of Breast Cancer Progression by Analysis of Genetic Markers," funded by the U.S. Army R&D Command ($88,056 for FY95).

"Vaccinia Virus Late Transcription," funded by the NIH ($16,000 for FY95).

"Detection of Morbillivirus Using a PCR-based Assay in Bottlenose Dolphins from the Atlantic and Gulf of Mexico Epizootics," funded by the Environmental Protection Agency ($55,530).

"Differential Display of Messenger RNAs Associated with Histologic Progression in Follicular Lymphomas," funded by the American Registry of Pathology ($15,400).

"Identification of the Virus Causing the 1918 Spanish Influenza Epidemic," Funded by the American Registry of Pathology ($9,880).

One grant in press.

PRESENTATIONS

1. Dr. Lichy presented a lecture entitled, "Novel Components of the Ras Signaling Pathway," at Howard University, Washington, D.C.
2. Dr. Taubenberger presented a lecture entitled, "The Role of LIP-6 in Hematopoiesis," for the Scientific Assembly, Alumni Reunion Weekend 1995, at the Medical College of Virginia, Richmond, Virginia,
3. Dr. Fanning presented a lecture entitled, "Transposable Elements in Cancer Cells," at the Roswell Park Cancer Institute in Buffalo, New York.
4. Dr. Tatro presented a lecture entitled, "Unmethylated LINE-1 Elements in Cancer Cells," at the mid-Atlantic transposable element meeting in Washington, D.C.

PUBLICATIONS

Journals

1. Krafft AE, Lichy JH, Lipscomb TP, Klaunberg, BA, Kennedy S, Taubenberger JK. Postmortem diagnosis of Morbillivirus infection in bottlenose dolphins (*Tursiops truncatus*) in the Atlantic and Gulf of Mexico epizootics by a polymerase chain reaction-based assay. *J Wildl Dis*. 1995;31:410-415.

2. Holmes KL, Lantz LM, Lee JS, Bedigian HG, Taubenberger, JK. Characterization of a new antigen expressed by B and myeloid lineage cells identified by the monoclonal antibody, LIP-6. *J Cell Immunol*. 1995;166:131-140.
3. Wright CF, Coroneos AM. The H4 subunit of vaccinia virus RNA polymerase is not required for transcription initiation at a viral late promoter. *J Virol*. 1995;69:3715-3720.
4. Alves G, Canavez F, Seuanez H, Fanning T. Recently amplified satellite DNA in Callithrix argentata (Primates, Platyrrhini). *Chromosome Res*. 1995;3:207-213.
5. Cushion M, Wright C, Tsai M, O'Leary TJ. Use of semiquantitative polymerase chain reaction to assess onset and treatment of *Pneumocystis carinii* infection in the rat model. *J Clin Microbiol*. 1995;33:718-724.
6. Hyman JA, Johnson LK, Tsai MM, O'Leary TJ. Specificity of polymerase chain reaction identification of *Toxoplasma gondii* infection in paraffin-embedded animal tissues. *J Vet Diagn Invest*. 1995;7:725-728.

One in press.

Abstracts

1. Carr NJ, Warren AY, Taubenberger JK, Lichy JH, Bratthauer GL, Sobin LH. Multiple squamous cell papillomas of the human esophagus unassociated with detectable human papillomavirus. *J Pathol*. 1995;176:53A.
2. Reid AH, Tsai MM, Venzon DJ, Wright CF, Lack EE, O'Leary TJ. mdm2 amplification, p53 mutation and 53 accumulation in malignant fibrous histiocytoma. United States and Canadian Academy of Pathology, Toronto, 1995. *Lab Invest*. 1995;72:11a.
3. Reid A, Edgerton S, Tsai M, Wright C, O'Leary T. p53 ccumulation and mutation are independent predictors of prognosis in node-positive breast cancer. United States and Canadian Academy of Pathology, Toronto, 1995. *Lab Invest*. 1995;72:24a.

In addition, eight papers and five abstracts are in press and four refereed papers have been submitted for publication.

DIVISION OF QUANTITATIVE PATHOLOGY

Robert L. Becker, Jr., Col, USAF, MC
Chief
Date of Appointment - 1 April 1988

MISSION

To conduct research and educational programs in flow cytometry, image analysis, morphometry, and artificial intelligence as applied to pathology. To develop applications of the above techniques for consultation, as appropriate.

STAFF

Medical

Robert L. Becker, Jr., Col, USAF, MC
William R. Oliver, MAJ, MC, USA

Scientific

Annette Geissel, HT (ASCP), Histology Technician
(D) Joe L. Griffin, Ph.D.
Lena Hohmann, B.S., Research Aid
(D) Ulrika V. Mikel, B.S., M.G.A., Research Cytologist
(A) Nina Sweeney, M.S., Computer Scientist

CONSULTATION

Cases

Description	*Reported*
Forensic	12
Flow/Image	74

The flow cytometry laboratory reported on 74 cases for DNA determination from paraffin-embedded material (an increase of more than 100% over the previous year). Most of these were specimens from molar pregnancies, where ploidy pattern helps to distinguish a partial mole from a complete mole or hydropic abortus. The image analysis laboratory consulted on 12 cases for image enhancement and analysis in support of the Office of the Armed Forces Medical Examiner.

RESEARCH

The flow cytometry and imaging laboratories were actively involved in intramural and extramural research projects during 1995.

A study relating flow and image-derived features to prognosis for prostatic carcinoma continued with the Department of Genitourinary Pathology and the U.S. Naval Hospital in San Diego. At year end, collection of image analysis raw data was complete from 104 of 128 cases. Features under study include DNA ploidy and S-phase determination by flow and image techniques, nuclear roundness (per Diamond et al.), prominence of neurosecretory differentiation (from immunohistochemistry), tumor grade, and tumor stage. For multifocal tumors, keyed subspecimens are being analyzed to capture any effect of tumor heterogeneity on the accuracy of outcome prediction. Almost all the cases processed thus far have yielded good to excellent DNA histograms for analysis. There are occasional ploidy differences between locations in the same resection specimen, or between flow and image ploidy determinations from the same nuclear preparation. Most tumors are diploid; the most commonly observed DNA content change is tetraploidy. There are frequent differences between flow and image methods in comparing proliferation of control and tumor areas, with an indication that image-based methods might be more specific in detecting elevated proliferation rate. These observations are preliminary, awaiting processing and analysis of the remaining 24 cases and correlation with clinical outcome.

We have further investigated methods for quantitative densitometric analysis of tissues stained for two components (i.e. DNA through variants of the Feulgen reaction and antigenic markers such as keratin or LINE-1 gene product through immunohistochemistry with diaminobenzidine as the chromogen). Preliminary results were presented at the 1995 USCAP meeting and at the 9th International Conference on Diagnostic Quantitative Pathology at Heidelberg, Germany. With our current technique, DNA histograms have been obtained from dual-stained specimens with resolution similar to that of histograms from cells stained only for DNA. The significance is that, for the first time, precise densitometric measurements can be obtained for more than one biologic marker in histologically localized cell populations. This method complements flow-cytometric or fluorescence-based imaging methods for multiparametric study of cells or tissues. Recent results suggest approaches to improve spectral resolution further in order to separate signals from two, or perhaps three, markers optimally.

During 1995, we completed two projects for three-dimensional reconstruction of cells and tissues.

The first, in collaboration with the Department of Genitourinary Pathology, concerned automated detection of hybridization sites for chromosome-specific probes in thick tissue sections. Three-dimensional data sets, obtained by confocal microscopy, were interactively analyzed using three-dimensional visualization software developed in our division. The per-cell chromosome counts from thick sections and confocal images were consistently higher with the confocal data sets than with thin sections examined by brightfield microscopy. We concluded that confocal data are superior for detecting focal aneusomy in small areas of histologic interest. The present method remains laborious; attempts to more fully automate it may be undertaken in 1996.

The second three-dimensional reconstruction project concerned renal glomerular vasculature. Working with rat glomeruli stained with eosin, we obtained confocal sections suitable for segmentation through the entire glomerular thickness. Dr. Elzbieta Kaczmarek, a Callender-Binford fellow from the University of Posnan, Poland, further refined our image acquisition methods to compensate for signal attenuation at deep focal planes and accomplished semi-automated segmentation of image stacks from several glomeruli. We completed construction of graphs (trees) representing vascular connections and estimated average length per vessel and total vascular length for several glomeruli. The results indicate a significantly lower total vascular length than has been previously reported using stereological measurements. These findings were presented at the 9th International Conference on Diagnostic Quantitative Pathology at Heidelberg, Germany, and a manuscript has been submitted for publication. The approach is intended for the study of developing and diseased glomeruli, to probe for vascular alterations that cannot be appreciated from standard brightfield or electron microscopy.

Results from work previously funded by AFIP and the American Registry of Pathology were the basis of a successful grant application to the Women's Health Initiative of the U.S. Army Medical Research and Development Command for the study of prognostic features concerning atypical endometrial hyperplasia and its coexistence with or progression to carcinoma. Technical progress in microscope automation for random field sampling was accomplished during 1995 in support of this study.

Ms. Sweeney completed software using wavelet transforms in conjunction with artificial neural networks for classification of human chromosomes from one-dimensional axial densitometric tracings. These methods will subsequently be extended to two- dimensional chromosome images and may serve as a prelude to the development of two- and three-dimensional shape-based diagnostics. She has submitted for publication a manuscript describing the mathematical properties of wavelet transforms and their uses in medical image processing.

Ms. Sweeney began development of software for automatic segmentation of clustered cell images in support of a cell densitometry package intended for use by nonspecialists. The package incorporates several segmentation methods, including the watershed algorithm and a variant of the gray-weighted distance transform.

MAJ Oliver continued research on two-dimensional patterned injury analysis and three-dimensional reconstruction and rendering of forensic images, with 11 presentations, 3 articles published, and 5 manuscripts submitted or in press in 1995.

MAJ Oliver continued collaboration with researchers at the University of North Carolina at Chapel Hill for construction of a repository for patterned injury data (RPID). The need for the repository comes from the lack of extensive and well-documented patterned injury case material for reference by general forensic pathologists working up new cases. The goal is to create a digital version of informative images and related information from the files of the Office of the Armed Forces Medical Examiner and other prominent forensic pathologists and to make that material available through a wide area network tool to assist in consultation. One manuscript was published on RPID in 1995.

MAJ Oliver continued a collaboration with U.S. Army Institute for Dental Research at Ft Meade, Md., using laser range-finding data for three-dimensional analysis of patterned injuries. The rangefinder

under development by USAIDR provides surface topography data over a wide range of scales. MAJ Oliver has worked with data from this instrument to build a three-dimensional demonstration of chain impressions on human skin. The work generated one published article, and further funding is being sought in collaboration with other federal agencies.

EDUCATION

In-house education centered on image-analysis training for two ARP fellows, Dr. Elzbieta Kaczmarek and Dr. Stefan Pambuccian. These are continuing training functions, interwoven with the research projects of both scientists.

Our extramural educational mission was met through invited lectures and talks at courses and meetings as detailed in the "Presentations" section below.

OTHER ACTIVITIES

Col Becker served on the AFIP Research Committee, chaired the Institute's Information Guidance Council, chaired the cytopathology subgroup of the Tricare Region I Joint Laboratory Working Group, served on the Pathology Information Management Software (PIMS) Committee, CAP Telepathology Committee, Ad Hoc Committee on Computer Uses Within AFIP, the CAP Imaging Committee, and the ARP Research Committee. He is on the editorial board of a new journal, *Cell Vision*. He served on the NIH Special Study Section for instrumentation meeting in July 1995.

MAJ Oliver chaired the AFIP Scientific Computing Subgroup of the Information Guidance Council. He is the DoD representative on the planning committee for a National Policy Workshop on Imaging Technology and the Investigation of Child Abuse and is on the Planning and Technical Committee of LE96—Emerging Technologies in Law Enforcement, 1996. He has begun to organize a newly authorized AFIP fascicle on patterned injury interpretation and analysis.

Ms. Mikel served as a laboratory preceptor for the department's week-long Diagnostic Exfoliative and Fine Needle Aspiration Cytology Course in June and continued service on the editorial board of *Analytical and Quantitative Cytology and Histology*.

Ms. Sweeney, along with MAJ Oliver, administers the mixed-platform local area network for Quantitative Pathology and provides Unix support for users in the Institute-wide Scientific Computing Group for network, imaging, and database applications.

GOALS

Based on results of intramural analyses, we will extend our flow cytometry consultation service in 1996 to offer breast carcinoma analysis for S-phase fraction. Depending on analyses for significance in multivariate survival models, FCM analysis of prostatic carcinoma ploidy and/or S-phase fraction might also be offered. We intend to establish an SOP manual that will allow the image analysis laboratory to stand for CAP inspection and accreditation.

In research, we will pursue current clinicopathologic studies to completion, with special emphasis on the flow/image prostatic carcinoma study, a confirmatory breast carcinoma image analysis study, and the funded atypical endometrial hyperplasia study. Further development of dual marker densitometry techniques is an important focus, since it is an opportunity for a novel and fundable contribution beyond simple correlation of disease features with outcome. Development of funding for new forensics and telemedicine projects also has a high priority, since these are a major area for future consultation activity.

There are currently no plans to offer formal courses in quantitative microscopy or forensic image analysis during 1996. This might change if an opportunity can be found to offer a course with little financial risk to the Institute or ARP. Offered course material must have novelty, as well as practicality, if it is to draw good attendance. As we consolidate the newer techniques used in our re-

search, short practicum courses are a likely focus for new educational efforts.

PRESENTATIONS

1. February 13, 1995: Seattle, Wash., American Academy of Forensic Sciences, "A Tutorial on Image Processing in Patterned Injury Analysis," W. R. Oliver, LTC, MC, USA.
2. February 15, 1995: Seattle, Wash., American Academy of Forensic Sciences, "Use of Three-dimensional range-finding in the 3D Evaluation of Patterned Injuries," W. R. Oliver, LTC, MC, USA, and B. Altschuler, Col, USAF, DC.
3. March 13, 1995: Rockville, Md., AFIP Forensic Dentistry Course. "Advanced Forensic Image Analysis," W. R. Oliver, LTC, MC, USA.
4. March 16, 1995: Toronto, Canada, United States and Canadian Academy of Pathology Short Course, "Quantitative Methods for Predicting Tumor Prognosis," T. J. O'Leary, M.D., Ph.D., and R.L. Becker, Col, USAF, MC.
5. March 1995: Washington, D.C., Armed Forces Institute of Pathology, "Image Processing in a Case of Child Abuse," W. R. Oliver, LTC, MC, USA.
6. May 1995: Georgetown University, Washington, D.C., "Forensic Image Processing and Analysis," W. R. Oliver, LTC, MC, USA.
7. June 1995: Washington, D.C., Armed Forces DNA Identification Laboratory, "Image Processing in Forensic Pathology," W. R. Oliver, LTC, MC, USA.
8. July 1995: Fairfax, Va., Northern Virginia Society for Radiology Technicians, "Image Processing in Analysis of Murder and Assault," W. R. Oliver, LTC, MC, USA.
9. October 6, 1995: Rockville, Md., AFIP Basic Forensic Pathology Course. "Forensic Imaging and Pattern Injury Analysis," W. R. Oliver, LTC, MC, USA.
10. October 11, 1995: Washington, D.C., Applied Imaging and Pattern Recognition, SPIE, "Three-dimensional Visualizatoin in Forensic Pathologic Examination," W. R. Oliver, LTC, MC, USA, and B. Altschuler, Col, USAF, DC.
11. October 11, 1995: Washington, D.C., Applied Imaging and Pattern Recognition, SPIE, "Rapid 3-D Video/Laser Sensing and Digital Archiving with Immediate On-scene Feedback for 3-D Crime Scene/Mass Disaster Data Collection and Reconstruction," B. Altschuler, Col, USAF, DC, W. R. Oliver, LTC, MC, USA, and M. Altschuler, Ph.D.
12. October 26, 1995: Heidelberg, Germany, 9th International Conference of Diagnostic Quantitative Pathology, "The Length of Capillaries Reconstructed from Confocal Consecutive Images," E. Kaczmarek and R. L. Becker, Col, USAF, MC.
13. October 26, 1995: Heidelberg, Germany, 9th International Conference of Diagnostic Quantitative Pathology, "Densitometric Measurements of Two Chromogens Through Decomposition of Images Taken at Two Wavelengths," R. L. Becker, Col, USAF, MC, U. V. Mikel, and E. Kaczmarek.
14. October 28, 1995: Heidelberg, Germany, 9th International Conference of Diagnostic Quantitative Pathology, "When is Routine Clinical Use of a Quantitative Pathology Technique Appropriate?" R. L. Becker, Col, USAF, MC
15. October 1995: Washington, D.C., AFIP Criminalistics Pathology Course, "Image Processing in Forensic Pathology," W. R. Oliver, LTC, MC, USA.

PUBLICATIONS

Journal Articles and Proceedings

1. Becker RL. Applications of neural networks in histopathology. *Pathologica*. 1995;87:246-254.
2. Oliver WR, Chancellor S, Symon J, Cullip T, Soltys M, Rosenman J, Hellman R, Gormley W. Three-dimensional visualization of bullet path: verification by computed radiography. *J Foren-*

sic Sci. 1995;40:321-324.

3. Oliver WR, Altschuler B. Image processing and 3D visualization in the interpretation of patterned injury of the skin. *Proceedings of Investigative and Trial Image Processing*, SPIE. 1995;2567:193-202.
4. Stotts D, Smith J, Jeffay K, Dewan P, Smith D, Oliver WR. Early experience with the repository for patterned injury data. *Proceedings of Invesigative and Trial Image Processing*, SPIE. 1995;2567:249-260.

Abstracts

1. Becker RL, Mikel UV, Kaczmarek E. Image decomposition for dual densitometry. *Lab Invest*. 1995;72:162A.
2. Oliver WR, Altschuler BR. Evaluation of three-dimensional range data for the examination of patterned injury. *Proc Am Acad Forensic Sci*. 1995:138.
3. Oliver WR. A tutorial on image processing for the examination of patterned injuries. *Proc Am Acad Forensic Sci*. 1995:139.